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Clostridium algidixylanolyticum sp. nov., a psychrotolerant, xylan-degrading, spore-forming bacterium

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A psychrotolerant *Clostridium* species was isolated from vacuum-packed, temperature-abused raw lamb. Colonies of this micro-organism on sheep-blood agar were circular with an entire margin, grey-white, translucent and β-haemolytic. Cells were single, tapered, motile rods. Elliptical subterminal spores were produced in the late stationary growth phase. Spores did not cause swelling of the maternal cells. The micro-organism was obligately anaerobic. In peptone yeast extract glucose starch (PYGS) broth at pH 7·0, the micro-organism grew optimally between 25·5 and 30·0 °C. The temperature range for growth was 2·5–32·2 °C. At 26 °C, the micro-organism grew optimally at pH 6·8 to 7·0. The pH range for anaerobic growth was 4·7–9·1. The micro-organism was saccharoclastic, hydrolysed starch and degraded xylan. The fermentation products formed in PYGS broth were acetate, formate, lactate, ethanol, butyrate, butanol, hydrogen and carbon dioxide. The G+C content of the DNA was 38·4 mol%. Phylogenetic analyses indicated that the strain belongs to cluster XIVa of the genus *Clostridium* (*sensu* Collins *et al.* 1994). The new strain differed from phylogenetically related clostridia in terms of cellular fatty acid composition, soluble protein profiles and phenotypic properties. On the basis of phenotypic and genotypic characterization data, the strain was assigned to a new species, namely *Clostridium algidixylanolyticum*. The type strain is strain SPL73^T (= DSM 12273^T).

Keywords: *Clostridium algidixylanolyticum*, psychrotolerance, meat spoilage

INTRODUCTION

The development of chilled storage and modern packaging technologies (including vacuum packaging), besides extending the shelf-life of meat, created a specific ecological niche for low-temperature-growing bacteria able to proliferate in oxygen-deprived conditions. The majority of the bacteria associated with vacuum-packed cuts of meat are thought to be derived from the exogenous environment of the slaughtered animal (Gill, 1979; Nottingham, 1982) and occur only on the meat surface. Direct carcass contamination occurs during dressing and evisceration (Anonymous, 1993); soil and animal faeces are considered to be the primary reservoirs for this contamination (Bell, 1997).

Clostridia are known to reside and grow in soil, in animal feed and on aerial plant surfaces (Lund, 1986; Ercolani, 1997). In temperate countries, the soil temperature generally favours the growth and survival of psychrotolerant (psychrophilic and psychrophilic), rather than mesophilic, clostridial species (Lund, 1986). Consequently, these low-temperature-growing species from soil or, through ingestion, from faecal sources may contaminate carcasses and thereby become part of the microflora present on vacuum-packed cuts of meat. Little is known, however, about populations of psychrotolerant clostridia with respect to either their primary source in the farm environment or their presence and diversity in vacuum-packed meats. Characteristics of recently described psychrotolerant *Clostridium* spp. associated with vacuum-packed meats (Collins *et al.*, 1992; Kalchayanand *et al.*, 1993;

Abbreviations: FAMEs, fatty acid methyl esters; ML, maximum-likelihood; MP, maximum-parsimony.

The GenBank accession number for the 16S rRNA sequence of *Clostridium algidixylanolyticum* strain SPL73^T is AF092549.

Lawson *et al*., 1994; Broda *et al*., 1996) indicate that this group of clostridia may contain metabolically and phylogenetically diverse organisms. During investigations of 'blown-pack' spoilage of vacuum-packed lamb, a psychrotolerant, xylan-degrading *Clostridium* species was isolated. The aim of this study was to characterize this previously undescribed micro-organism and to determine if it could be differentiated from other psychrotolerant clostridia.

METHODS

Bacteria and culturing. Stringent anaerobic techniques (Holdeman *et al*., 1977) were used for media preparation and the culturing of all *Clostridium* strains. The handling of micro-organisms and the inoculation of test media were conducted inside an anaerobic chamber (Forma Scientific).

Strain SPL73^T (T = type strain) had been isolated from a 'blown', temperature-abused vacuum pack of raw lamb, using methods described previously (Broda *et al*., 1996). This meat-derived strain was revived from freeze-dried culture in peptone yeast extract glucose starch (PYGS) broth (Lund *et al*., 1990) and subcultured onto Columbia Blood Agar (CBA; Oxoid) containing 5% (v/v) sheep blood. These agar plates were incubated at 26 °C for 72 h.

Reference strains of *Clostridium xylanolyticum* (DSM 6555^T), *Clostridium aerotolerans* (DSM 5434^T), *Clostridium sphenoides* (DSM 632^T) and *Clostridium celerecrescens* (DSM 5628^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. Reference strains were revived from freeze-dried material in PYGS broth and plated on to CBA supplemented with 5% (v/v) sheep blood. All reference strains were grown at 37 °C for 48 h.

Phenotypic characterization. Colony morphology was described for strain SPL73^T grown on the surface of CBA with 5% (v/v) sheep blood at 26 °C for 72 h. The morphology and ultrastructure of vegetative cells and spores were determined for the strain after growth in PYGS broth at 26 °C for 48 h and 3 months, respectively. Vegetative cell and spore morphology was determined using a phase-contrast light microscope (Leitz Orthoplan). Cell- and spore ultrastructure was examined using a transmission electron microscope (Philips EM 400), as described previously (Broda *et al*., 1999). The presence of flagella was determined using a negatively stained 48-h PYGS broth culture and the transmission electron microscopy.

Vegetative cells of strain SPL73^T were Gram-stained using the method of Johnson *et al*. (1995) and the Gram type was also determined using a KOH test (Powers, 1995). The cell wall type was determined using transmission electron microscopy.

Physiological tests were conducted in anaerobic peptone yeast extract (PY) broth (Holdeman *et al*., 1977) with the glucose concentration adjusted to 0·5% (w/v). Physiological tests were performed, as described previously (Broda *et al*., 1999), in Hungate tubes containing broth inoculated with 2% (v/v) exponentially growing culture. The potential for growth at 20 pH values ranging from 4·5 to 9·2 was determined at 26 °C, the pH of the medium being kept constant by the addition of sterile, anaerobic potassium phosphate buffer. Growth rates at pH 7·0 were obtained for 42 temperatures ranging from −1·5 to 47·0 °C.

Substrate utilization for strain SPL73^T was tested anaerobically at 26 °C and an initial pH of 7·0. This biochemical test was conducted according to Holdeman *et al*. (1977) in PY broth containing 0·1% (w/v) yeast extract for the range of substrates described previously (Broda *et al*., 1999). Fermentation products were determined for strain SPL73^T grown in PYGS broth at 26 °C for 10 d. Ether extracts of volatile fatty acids and alcohols, and methyl esters of non-volatile fatty acids, were prepared according to Holdeman *et al*. (1977) and analysed as described previously (Broda *et al*., 1999).

Strain SPL73^T was tested for toxicity in mice using procedures described in the *Bacteriological Analytical Manual* of the Food & Drugs Administration (Solomon *et al*., 1995).

Xylanolytic activity. The ability of strain SPL73^T to degrade xylan was initially determined on PY agar containing 3% (w/v) oat-spelt xylan (Sigma) after 72 h incubation at 26 °C. To determine xylanase activity secreted into culture supernatant, the strain was grown at 26 °C for 72 h in PY broth supplemented with 0·5% (w/v) oat-spelt xylan. A cell-free culture supernatant was prepared by centrifugation of the culture at 12000 ***g*** for 15 min subsequent sonication of the supernatant and then further centrifugation. Xylanase activity was measured at both pH 5·6 and pH 7·0 by determining reducing-sugar (xylose) release. Enzyme activity at each pH was tested in the reaction mixtures containing culture supernatant, oat-spelt xylan and potassium phosphate buffer. The final concentrations of xylan and potassium phosphate in each mixture were 1% (w/v) and 80 mM, respectively. These reaction mixtures were incubated at 26 °C for 1 h. The reducing sugar formed was measured using the dinitrosalicilic acid method (Miller, 1959). One unit of xylanase activity secreted in 1 ml culture supernatant was defined as the activity that released 1 μmol xylose in 1 min.

16S rRNA sequence determination and phylogenetic analysis. For 16S rRNA sequence determination, strain SPL73^T was grown in PYGS broth at 25 °C for 48 h. DNA was isolated using the method of Marmur (1961), modified as described by Johnson (1994). The G+C content (mol%) of the DNA from the strain was determined according to the method of Mesbah *et al*. (1989).

The 16S rRNA sequence was determined from the PCR-amplified 16S rDNA fragment (Broda *et al*., 1999). The PCR fragment was purified with the Wizard purification kit (Promega, Dade Diagnostics), according to the manufacturers' instructions, and sequenced directly in an ABI 377 automated sequencer (Perkin-Elmer, Applied Biosystems). Forward- and reverse sequences of the 16S rDNA gene were obtained using sequencing conditions and primers described previously (Broda *et al*., 1999). The sequences were aligned using Sequence Navigator (Perkin-Elmer) and corrected manually in agreement with the electropherograms and the reading of complementing and overlapping fragments. Preliminary sequence analysis was performed on Silicon Graphics Indigo 2 using the GCG package (Devereux *et al*., 1984). Sequences were aligned using the program PILEUP (Feng & Doolittle, 1987) with low gap weighting and low gap extension weighting. There was little length polymorphism between the sequences selected for the phylogenetic analysis and so the alignment was unequivocal and no manual adjustments were required. A total of 1395 characters of the aligned sequences were used for the phylogenetic reconstruction, with gaps being treated as missing data. Phylogenetic analysis was performed with

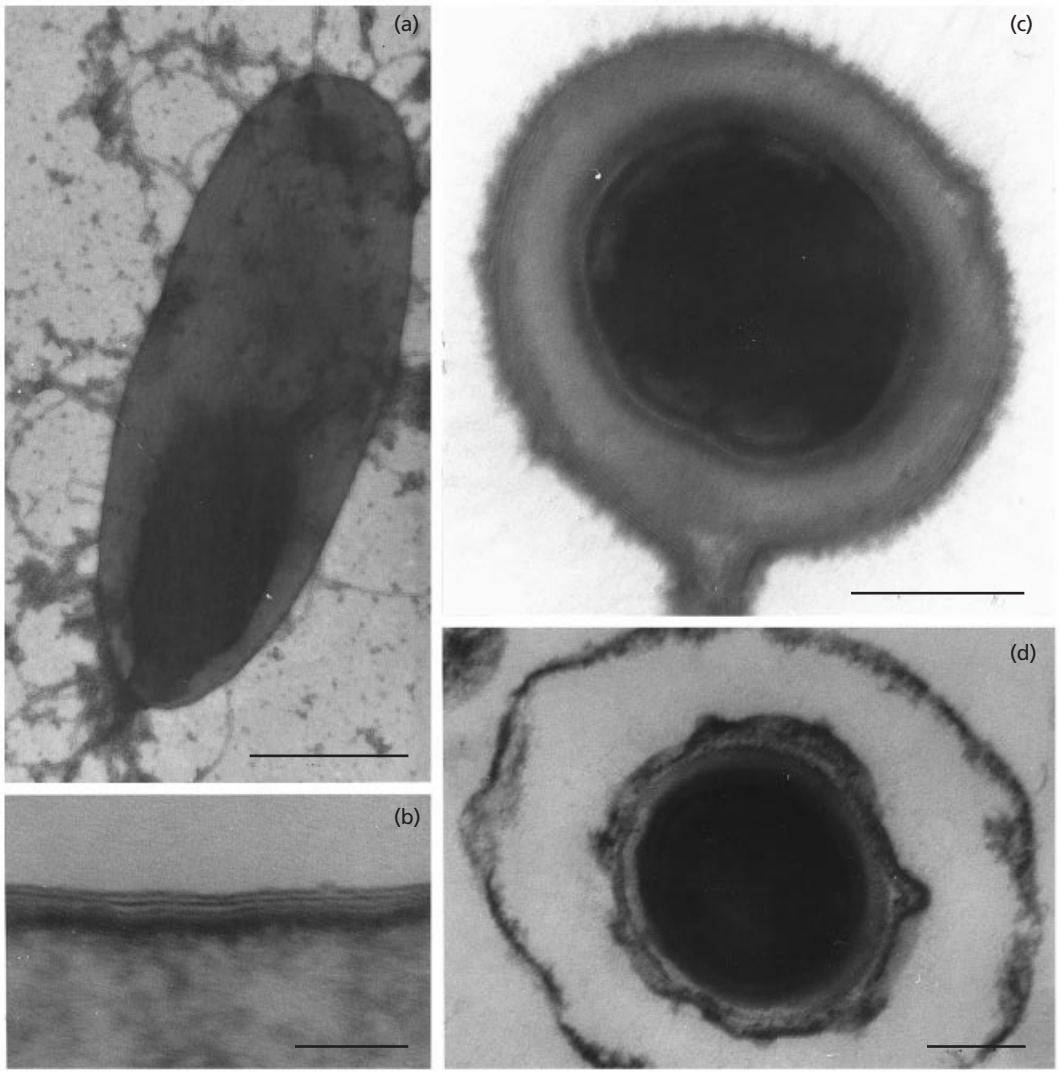

Fig. 1. Electron micrographs of cells of strain SPL73^T showing peritrichous flagella in a sporulated cell (a), the multilayer cell wall (b), a thin section of a sporulated cell (c) and a thin section of a mature spore (d). Bars, 0·5 μm (a), 0·1 μm (b) and 0·3 μm (c, d).

both maximum-parsimony (MP) and maximum-likelihood (ML) using the program PAUP* (Swofford, 1996). MP-based analyses were performed using a branch and bound search. For MP bootstrap analysis, 2000 heuristic replicate analyses were carried out, each with 10 random sequence additions. ML-based analyses were performed using the HKY85 (Hasegawa *et al.*, 1985) substitution model. Rates were assumed to follow a gamma distribution and the gamma shape parameter and transition to transversion ratios were estimated from the most parsimonious tree. ML bootstrap analysis was performed using the same model with 100 heuristic replicates.

Relatedness to phylogenetically related clostridia. The relatedness of strain SPL73^T to phylogenetically related clostridia was assessed using SDS-PAGE of total soluble cell proteins and also by means of cellular fatty acid analysis.

Samples and a high-range protein standard (Bio-Rad) for SDS-PAGE were prepared as described previously (Broda *et al.*, 1999). SDS-PAGE was performed using the discontinuous buffer system of Laemmli (1970) in either 8–16% gradient Tris/HCl or 10% Tris/HCl polyacrylamide minigels (Bio-Rad) in a Ready Gel Cell vertical gel apparatus (Bio-Rad). Electrophoresis was carried out at constant voltage of 150 V for 1 h and the gel was stained with Coomassie blue R-250 (BDH).

For cellular fatty acid analysis, fatty acid methyl esters (FAMEs) were extracted as described by Kuykendall *et al.* (1988). The C10 to C20 fatty acid identification and quantification was performed using a Hewlett Packard 6890 gas chromatograph, as described previously (Broda *et al.*, 1999).

RESULTS

Phenotypic characterization

The colonies of strain SPL73^T grown on the surface of CBA containing 5% sheep blood were circular, 0·8–2·5 mm in diameter, possessed entire margins and were

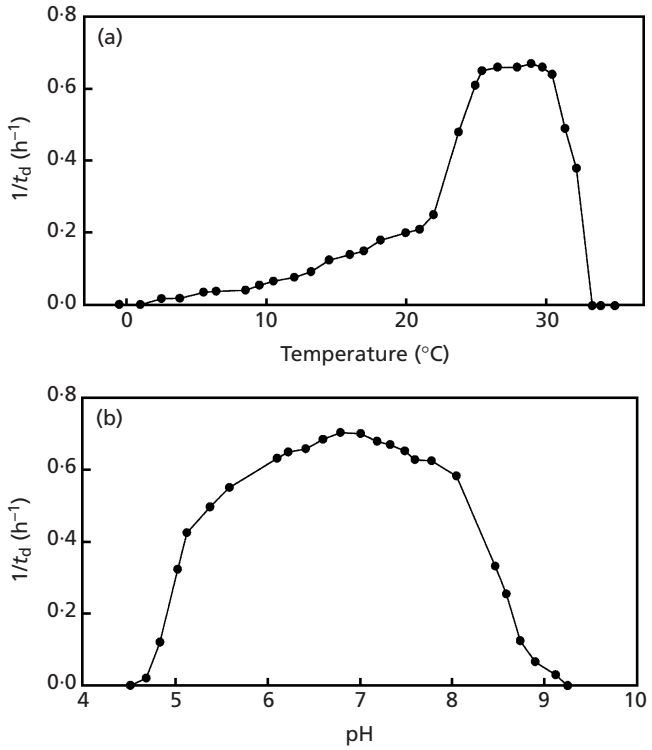

Fig. 2. Growth of strain SPL73^T at various incubation temperatures at pH 7·0 (a) and at various pH values at 26 °C (b). t_d, Doubling time.

greyish white, translucent, raised, convex, shiny and β-haemolytic. Cells in the exponential growth phase occurred singly as tapered straight to slightly bent rods 1·8–2·8 μm long and 0·5–0·8 μm wide. Both non-sporulated and sporulated cells were motile by means of peritrichous flagella (Fig. 1a). Electron micrographs of ultrathin sections of vegetative cells confirmed that the organism was rod-shaped. The multilayer cell wall was observed in ultrathin sections of vegetative cells of the strain (Fig. 1b). Conventional Gram-staining and the KOH reaction were indicative of a Gram-negative cell wall type. During the late stationary growth phase, elliptical, subterminal spores were formed (Fig. 1c). The mature spores had a fully developed endospore structure (Fig. 1d). Spores did not swell the maternal cells.

No growth of strain SPL73^T occurred on aerobic CBA containing 5% sheep blood. Strain SPL73^T grew optimally anaerobically between 25·5 and 30·0 °C (Fig. 2a). The lowest and highest temperatures at which growth of this strain was observed were 2·5 and 32·2 °C, respectively. The optimal pH for anaerobic growth was 6·8–7·0 and growth occurred at pH values between 4·7 and 9·1 (Fig. 2b).

Strain SPL73^T was saccharoclastic, but not proteolytic. Little, if any, growth of the meat strain occurred in media containing peptone and yeast extract but lacking added carbohydrate. Similarly, only little growth of strain SPL73^T occurred in basal medium (Holdeman *et al.*, 1977) containing carbohydrate but lacking peptone, tryptone and yeast extract. In PY broth without yeast extract, the meat strain reached about half the level of turbidity produced in broth supplemented with yeast extract. The substrates fermented by strain SPL73^T in PY broth were arabinose, cellobiose, fructose, galactose, glucose, inulin, lactose, maltose, mannose, rhamnose, raffinose, salicin, sucrose and xylose. The substrates not fermented were adonitol, dextran, inositol, mannitol, sorbitol, sorbose and trehalose. Starch was hydrolysed and hydrogen sulphide was produced. Gelatin and aesculin were not hydrolysed, lecithinase and lipase activities were absent, meat and casein were not digested and the milk reaction was negative. Neither indole nor ammonia was produced, nitrate was not reduced, oxidase activity and catalase activity were absent and urea was not hydrolysed. No growth was observed in the presence of bile. The addition of Tween 80 did not stimulate growth. The major fermentation products formed by strain SPL73^T in PYGS broth were acetate, formate, lactate, ethanol, butyrate, butanol, hydrogen and carbon dioxide.

The supernatant of the broth culture of strain SPL73^T was not toxic to mice.

Xylanolytic activity

On medium containing 3% oat-spelt xylan, colonies of strain SPL73^T were surrounded by clear zones indicative of xylan degradation. At 26 °C, the xylanase activity secreted into the culture supernatant of this strain was 0·110 U ml^{-1} at pH 7·0 and 0·073 U ml^{-1} at pH 5·6.

16S rRNA sequence determination and phylogenetic analysis

The G+C content of the DNA of strain SPL73^T was 38·4 mol%.

The 16S rRNA sequence of strain SPL73^T containing a continuous stretch of 1502 nucleotides (approximately positions 8–1518 according to *Escherichia coli* numbering) was used to search the GenBank and Ribosomal Database Project libraries. Sequence searches showed that strain SPL73^T was phylogenetically most closely associated with the *Clostridium* subphylum (data not shown). The results of the sequence similarity calculations indicated that the nearest relatives of strain SPL73^T are *C. aerotolerans* (97·7%), *C. xylanolyticum* (97·6%), *C. celerecrescens* (97·0%) and *C. sphenoides* (96·3%).

Of the aligned 1395 characters there were 152 parsimony-informative sites. The search using MP as an optimality criterion generated a single most parsimonious tree of length 487. The consistency index of

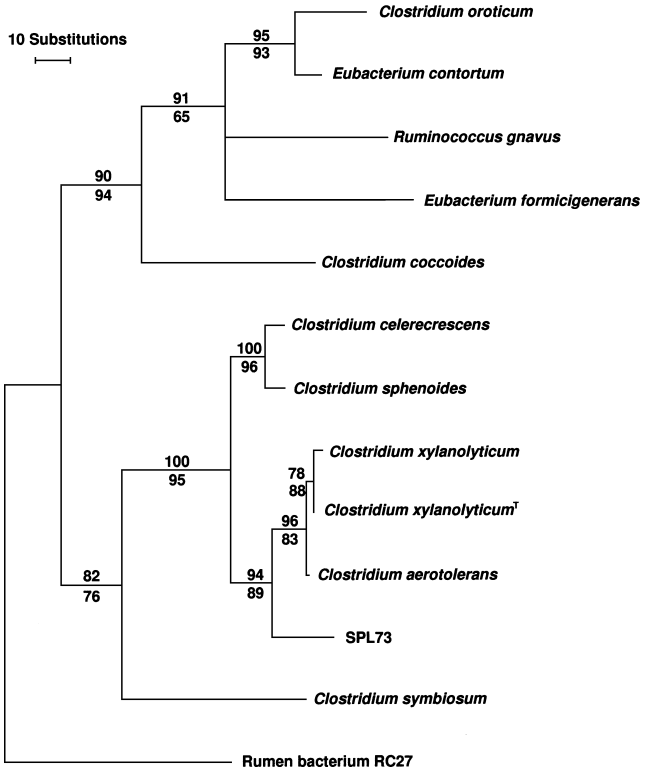

Fig. 3. Consensus maximum-parsimony and maximum-likelihood tree generated from aligned 16S rRNA gene sequences. The values above branches indicate the level of support derived from maximum-parsimony bootstrap analyses of 2000 replicates. The values below branches indicate the level of support derived from maximum-likelihood bootstrap analyses of 100 replicates. The following reference strains were used in phylogenetic analyses: *Clostridium aerotolerans* DSM 5434^T (X76163), *Clostridium celerecrescens* DSM 5628^T (X71848), *Clostridium coccoides* DSM 2088 (M59090), *Clostridium oroticum* ATCC 13619^T (M59109), *Clostridium sphenoides* DSM 632^T (X73449), *Clostridium symbiosum* ATCC 14940^T (M59112), *Clostridium xylanolyticum* DSM 6555^T (X71855), *Clostridium xylanolyticum* ATCC 4963 (X76739), *Eubacterium contortum* ATCC 25540^T (L34615), *Eubacterium formicigenerans* ATCC 27755^T (L34619) and *Ruminococcus gnavus* ATCC 29149^T (L76597). Unidentified rumen bacterium RC27 (AF001716) was used as an outgroup. Bar, 10 nucleotide substitutions.

this tree was 0·690 and the retention index was 0·659. The ML tree differed from the MP tree in the branching order of *Ruminococcus gnavus* and *Eubacterium formicigenerans*. This difference, however, did not affect the phylogenetic position of strain SPL73^T. The consensus MP and ML tree is shown in Fig. 3.

Detailed phylogenetic analyses placed strain SPL73^T within cluster XIVa of the genus *Clostridium* (*sensu* Collins *et al*. 1994). In this cluster, strain SPL73^T was located within a unit composed of *C. xylanolyticum* and *C. aerotolerans*. Within this unit, however, strain SPL73^T represented a distinct branching line. The robustness of this grouping was well supported by the results of both MP and ML bootstrap analyses (94 and 89 %, respectively).

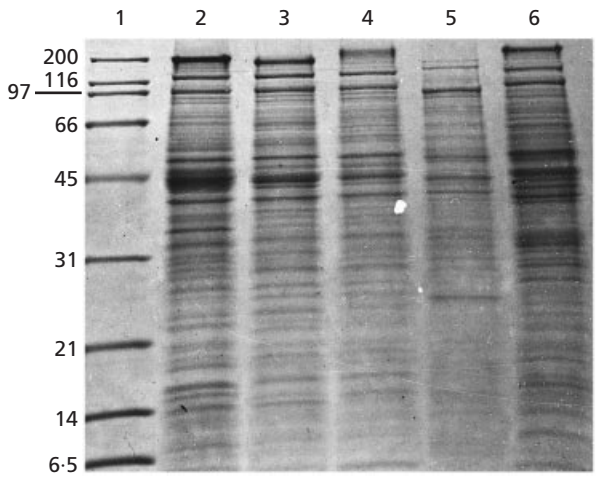

Fig. 4. SDS-PAGE of soluble cell proteins from strain SPL73^T and phylogenetically related clostridia. Electrophoresis was carried out in 8–16 % gradient Tris/HCl polyacrylamide gel. Lanes: 1, broad-range protein standard; 2, strain SPL73^T; 3, *C. xylanolyticum* (DSM 6555^T); 4, *C. aerotolerans* (DSM 5434^T); 5, *C. celerecrescens* (DSM 5628^T); 6, *C. sphenoides* (DSM 632^T). The values on the left of lane 1 indicate the positions of protein standards (kDa).

Relatedness to phylogenetically related clostridia

Gel electrophoresis of soluble cell proteins demonstrated that there were distinct differences between the profiles of meat strain SPL73^T and those of its phylogenetic relatives *C. xylanolyticum* (DSM 6555^T), *C. aerotolerans* (DSM 5434^T), *C. sphenoides* (DSM 632^T) and *C. celerecrescens* (DSM 5628^T). The main dissimilarities between these profiles are differences in the concentrations and positions of major protein bands at approximately 45 kDa and of minor bands (Fig. 4).

The major fatty acids detected in the cell extract of strain SPL73^T were myristic (C14:0, 3·9 %), palmitic (C16:0, 17·8 %), palmitoleic (C16:1, 6·6 %) and oleic (C18:1, 5·0 %) acids. Distinct differences were observed between the cellular fatty acid pattern of the meat strain and the patterns of its phylogenetic neighbours (Table 1). The cellular fatty acid pattern of *C. xylanolyticum* (DSM 6555^T) differed from the pattern of strain SPL73^T by the presence of stearic acid (C18:0) and three unknown compounds (equivalent chain lengths 15·672, 17·633 and 18·475). In addition, the cellular fatty acid composition of *C. xylanolyticum* (DSM 6555^T) differed from that of the meat strain in the proportion of palmitic (C16:0) and palmitoleic (C16:1) acids formed. The cellular fatty acid pattern of *C. aerotolerans* (DSM 5434^T) contained only two fatty acids, contrasting with the eight associated with the meat strain. The pattern of *C. sphenoides* (DSM 632^T) contained a higher proportion of palmitic acid (C16:0) and a lower proportion of palmitoleic acid (C16:1) relative to strain SPL73^T. The pattern of *C. sphenoides*

Table 1. Differences in cellular fatty acid composition of strain SPL73^T and phylogenetically related clostridia (values are percentage of total peak area)

1, SPL73^T; 2, *Clostridium xylanolyticum* DSM 6555^T; 3, *Clostridium aerotolerans* DSM 5434^T; 4, *Clostridium sphenoides* DSM 632^T; 5, *Clostridium celerecrescens* DSM 5628^T.

Equivalent chain length	FAME*	1	2	3	4	5†
14·000	14:0	3·9	4·3	—	2·4	9·2
15·350	Unknown	3·5	—	—	1·9	—
15·672	Unknown	—	3·5	—	1·8	1·2
16·000	16:0	17·8	3·0	19·6	30·3	14·0
16·398	16:1	6·6	1·7	—	3·9	1·0
17·538	Unknown	9·2	12·6	8·3	6·8	3·2
17·633	Unknown	—	5·5	—	6·1	—
18·000	18:0	—	4·1	—	4·5	5·5
18·362	18:1	5·0	3·1	—	6·0	5·6
18·404	Unknown	6·3	5·4	—	2·7	—
18·493	Unknown	3·0	—	—	6·8	—
18·475	Unknown	—	2·6	—	2·9	1·9

*Only C10 to C20 fatty acids are listed. Concentrations of individual fatty acids are expressed as the percentage of total peak area that might have included peaks of straight chain and/or branched fatty acids with an effective chain length of less than 10, as well as some unknown compounds.

†For *C. celerecrescens*, only fatty acids occurring in concentrations over 1 % are listed. With this strain, a number of fatty acids were also detected at concentrations below 1 %. Because these fatty acids did not occur in the remaining strains, they have been omitted from this table because of low comparative values.

also contained stearic acid (C18:0) and three unknown compounds (equivalent chain lengths 15·672, 17·633 and 18·475), which were absent from the pattern for the meat strain.

DISCUSSION

The results of 16S rRNA sequence similarity analysis and database searches show that strain SPL73^T was most similar to *C. aerotolerans* (DSM 5434^T), *C. xylanolyticum* (DSM 6555^T), *C. celerecrescens* (DSM 5628^T) and *C. sphenoides* (DSM 632^T). The results of phylogenetic analyses demonstrate, however, that the meat strain represents a distinct branch within a phylogenetic unit containing two strains of *C. xylanolyticum* and *C. aerotolerans* (Fig. 3). The meat strain characterized in this study was readily distinguished from its phylogenetic neighbours by its soluble protein profiles and cellular fatty acid profiles. In addition, strain SPL73^T differed from its phylogenetic neighbours in terms of phenotypic characteristics (Table 2). The phylogenetic relatives of strain SPL73^T are mesophiles and grow optimally at temperatures between 30 and 38 °C (Cato *et al.*, 1986; van Gylswyk & van der Toorn, 1987; Palop *et al.*, 1989; Rogers & Baecker, 1991), whereas the meat strain is unable to grow at temperatures above 32·2 °C and grows optimally between 25·5 and 30·0 °C. In contrast to *C. aerotolerans*, strain SPL73^T was obligately anaerobic. Additional phenotypic properties that differentiate strain SPL73^T from *C. xylanolyticum* and *C. aerotolerans* were the fermentation of arabinose and lactose (unlike *C. xylanolyticum* the meat strain ferments these two carbohydrates) and the fermentation of inulin and trehalose (in contrast to *C. aerotolerans*, the meat strain ferments inulin but not trehalose) (van Gylswyk & van der Toorn, 1987; Rogers & Baecker, 1991). Unlike strain SPL73^T, its two other nearest phylogenetic neighbours, *C. sphenoides* and *C. celerecrescens*, produce indole and ferment a different range of carbohydrates (Cato *et al.*, 1986; Palop *et al.*, 1989).

The results of detailed phylogenetic analyses conducted in this study placed strain SPL73^T in cluster XIVa of the genus *Clostridium* (*sensu* Collins *et al.* 1994). Members of cluster XIVa are phylogenetically distinct from *sensu stricto* clostridia belonging to cluster I (Collins *et al.*, 1994), i.e. rRNA homology group I (Johnson & Francis, 1975). Cluster XIVa contains phenotypically and phylogenetically heterogeneous micro-organisms that include non-spore-forming cocci (e.g. ruminococci), aerotolerant rods (e.g. *C. aerotolerans*) and other organisms that frequently have one or more characteristic(s) that conflicts with those defined for members of the genus *Clostridium* (Cato *et al.*, 1986). Future taxonomic reclassification of this cluster, therefore, is likely to define a number of new genera and may result in nomenclatural changes for clostridial species currently contained within the cluster. However, until a taxonomic review of the genus *Clostridium* is undertaken, strain SPL73^T can be assigned to the genus *Clostridium*.

The majority of known psychrotolerant *Clostridium* spp. appear to form a very diverse group of dominantly Gram-positive, low-G+C organisms belonging to cluster I clostridia (Collins *et al.*, 1994). Strain SPL73^T, with its multilayer thin-section profile of cell wall, its G+C content of 38·4 mol % and its phylogenetic placement in cluster XIVa, can be readily differentiated from non-proteolytic *Clostridium botulinum* types B, E and F (Cato *et al.*, 1986), *Clostridium estertheticum* (Collins *et al.*, 1992), *Clostridium algidicarnis* (Lawson *et al.*, 1994), *Clostridium vincentii* (Mountfort *et al.*, 1997), *Clostridium frigidicarnis* (Broda *et al.*, 1999) and *Clostridium gasigenes* (Broda *et al.*, 2000). This meat strain differs from *Clostridium arcticum* (which stains Gram-negative and is not yet characterized with respect to G+C content and phylogenetic placement) and from *Clostridium fimetarium* (which has a G+C content of 35·6 mol % and also lacks phylogeny data) by its ability to hydrolyse starch and ferment lactose, maltose, raffinose, rhamnose and sucrose (Jordan & McNicol, 1979; Kotsyurbenko *et al.*, 1995). In ad-

Table 2. Some properties by which strain SPL73^T can be differentiated from phylogenetically related clostridia

1, SPL73^T; 2, *C. xylanolyticum* DSM 6555^T; 3, *C. aerotolerans* DSM 5434^T; 4, *C. sphenoides* DSM 632^T; 5, *C. celerecrescens* DSM 5628^T. Results in parentheses are for some strains of the species. +, Positive; −, negative; w, weak reaction (at pH 5·5–5·9); d, 40–60 % of strains positive; ±, 61–89 % of strains positive; NR, not reported; A, acetate; B or b, butyrate; c, caproate; F or f, formate; ib, isobutyrate; iv, isovalerate; L or l, lactate; s, succinate; 2, ethanol; 4, butanol. Upper-case letters indicate major fermentation products and lower-case letters indicate minor fermentation products.

Phenotypic property	1	2*	3†	4‡	5§
Maximum growth temp. (°C)	32·2	NR	<45	<45	NR
Optimum growth temp. (°C)	25·5–30·0	35	38	30–37	35
Fermentation products	A,F,L,b,2,4	F,L,A,2	A,F,L,2	A,2 (F,s,l)	A,F,B,ib,iv, c,l,s,2
Gelatin hydrolysis	−	−	−	−	+
Indole production	−	−	−	+	+
Nitrate reduction	−	−	−	±	−
Fermentation of:					
Arabinose	+	−	+	−w	+
Inulin	+	NR	−	−	−
Lactose	+	−	+	+w	+w
Trehalose	−	−	d	d	+

* Results are from Rogers & Baecker (1991).

† Results are from van Gylswyk & van der Toorn (1987).

‡ Results are from Cato *et al.* (1986).

§ Results are from Palop *et al.* (1989).

dition, strain SPL73^T is unable to produce indole, whereas *C. arcticum* is able to do so, and strain SPL73^T ferments arabinose, whereas *C. arcticum* does not. Unlike strain SPL73^T, *Clostridium putrefaciens* is non-saccharolytic (Cato *et al.*, 1986) and *Clostridium laramiense* reduces nitrate, has lipase activity and ferments a different range of carbohydrates (Kalchayanand *et al.*, 1993; Trüper & de' Clari, 1997).

The common sources of xylan-fermenting clostridia are farm soil, animal feeds and wood chips (Lamed & Zeikus, 1980; van Gylswyk & van der Toorn, 1987; Rogers & Baecker, 1991). Xylanolytic clostridia (e.g. *Clostridium polysaccharolyticum*, *C. aerotolerans*) may also be present in the rumen. However, as strain SPL73^T cannot proliferate at temperatures above 33 °C, well below ruminant body temperature, the source of carcass contamination with this meat strain appears to be extrinsic. Such contamination may occur as a result of contact either with spores that survive passage through the digestive system of slaughter animals or with spores of environmental origin that are carried on the coats of slaughter stock. Alternatively, contamination may occur from a secondary source located within the meat plant, such as the ventilation- or drainage systems.

Results of morphological, biochemical and physiological characterization conducted in this study consistently demonstrate distinct differences between strain SPL73^T and its nearest taxonomic neighbours. Both the chemotaxonomic characterization data and the unique phylogenetic position of strain SPL73^T confirm the distinctiveness of the meat strain relative to currently described clostridial species, fulfilling the requirements for polyphasic delineation of bacterial taxa. Therefore, strain SPL73^T merits classification as a new species within the genus *Clostridium*. For this species, we propose the name *Clostridium algidixylanolyticum*.

Description of *Clostridium algidixylanolyticum* sp. nov.

Clostridium algidixylanolyticum (al.gi.di.xy.la.no.ly′ti.cum. L. adj. *algidus* cold; Gr. derived M.L. n. *xylanum* xylan; Gr. adj. *lyticus* dissolving; N.L. gen. n. *algidixylanolyticum* cold xylan-dissolving).

Colonies on sheep-blood agar are 0·8–2·5 mm in diameter, circular with an entire margin, greyish white, translucent and *β*-haemolytic. Cells are single, tapered, motile rods (1·8–2·8 μm long and 0·5–0·8 μm wide). Elliptical spores do not swell maternal cells and are produced in the late stationary growth phase. Obligately anaerobic. At pH 7·0, the micro-organism grows optimally between 25·5 and 30·0 °C. At 26 °C, it grows optimally at pH 6·8–7·0. Temperature range for

growth is 2·5–32·2 °C; pH range for growth is 4·7–9·1. Saccharoclastic and ferments arabinose, cellobiose, fructose, galactose, glucose, inulin, lactose, maltose, mannose, raffinose, rhamnose, salicin, sucrose and xylose. Starch is hydrolysed and xylan is degraded. Fermentation products formed in PYGS broth are acetate, formate, lactate, ethanol, butyrate, butanol, hydrogen and carbon dioxide. The supernatant of the broth culture of the micro-organism is non-toxic to mice. G+C content of the DNA is 38·4 mol%. Isolated from vacuum-packed, temperature-abused raw lamb. The type strain is strain SPL73^T (= DSM 12273^T).

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